



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁵ : C12P 21/06, C12Q 1/68 C07K 3/00, 13/00, 15/00 C07K 17/00, G01N 33/566</p>	<p>A1</p>	<p>(11) International Publication Number: WO 93/06229 (43) International Publication Date: 1 April 1993 (01.04.93)</p>
<p>(21) International Application Number: PCT/US92/07641 (22) International Filing Date: 14 September 1992 (14.09.92) (30) Priority data: 759,568 13 September 1991 (13.09.91) US (71) Applicant: THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Box OTT, Bethesda, MD 20892 (US). (72) Inventor: MURPHY, Philip, M. ; 987 Paulsboro Drive, Bethesda, MD 20892 (US). (74) Agents: SCOTT, Watson, T. et al.; Cushman, Darby & Cushman, 1100 New York Avenue, N.W., Washington, DC 20005 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published With international search report.</p>
<p>(54) Title: CLONING OF cDNA ENCODING A FUNCTIONAL HUMAN INTERLEUKIN-8 RECEPTOR (57) Abstract A cDNA clone from HL60 neutrophils, designated p2, which encodes a human interleukin-8 receptor. This IL-8 receptor can be expressed in oocytes or transfected host cells. This receptor has 77 % amino acid identity with a second human neutrophil receptor isotype that also binds IL-8. It also exhibits 69 % amino acid identity with a protein reported to be an N-formyl peptide receptor from rabbit neutrophils.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	MN	Mongolia
AU	Australia	FR	France	MR	Mauritania
BB	Barbados	GA	Gabon	MW	Malawi
BE	Belgium	GB	United Kingdom	NL	Netherlands
BF	Burkina Faso	GN	Guinea	NO	Norway
BG	Bulgaria	GR	Greece	NZ	New Zealand
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	PT	Portugal
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovak Republic
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CS	Czechoslovakia	LU	Luxembourg	SU	Soviet Union
CZ	Czech Republic	MC	Monaco	TD	Chad
DE	Germany	MG	Madagascar	TC	Togo
DK	Denmark	ML	Mali	UA	Ukraine
ES	Spain			US	United States of America

CLONING OF CDNA ENCODING A FUNCTIONAL
HUMAN INTERLEUKIN-8 RECEPTOR

BACKGROUND OF THE INVENTION

Field of the Invention

5 The invention relates to identification and characterization of a human interleukin-8 receptor which also binds gro. In another aspect, it relates to stable expression of functionally active IL-8 receptor in host cells.

10 Background Information

 Stimulation of neutrophils with IL-8, NAP-2 or gro causes mobilization of intracellular calcium stores and elicits motile, secretory, and metabolic responses that are critical to the role of the
15 neutrophil in host defenses. See B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggiolini, J. Exp. Med. 171, 1797 (1990); A. Walz, B. Dewald, V. von Tschanner, M. Baggiolini, ibid. 170, 1745 (1989); and M. Thelen et al., FASEB J. 2, 2702 (1988).

20 IL-8 is an inflammatory cytokine that activates neutrophil chemotaxis, degranulation and the respiratory burst, the means by which neutrophils attack pathogens in the body. Neutrophils express receptors for IL-8 that are coupled to guanine
25 nucleotide binding proteins (G-proteins); binding of IL-8 to its receptor induces the mobilization of intracellular calcium stores. IL-8, also known as

neutrophil activating protein-1 or NAP-1, is a potent chemoattractant for neutrophils that is produced by many cell types in response to inflammatory stimuli. See J. J. Oppenheim, Prog. Clin. Biol. Res. 349, 405 (1990). This IL-8 receptor has 77% amino acid identity with a second human neutrophil receptor isotype that also binds IL-8 (Genentech, FASEB, April 1991).

IL-8 is structurally and functionally related to several members of the macrophage inflammatory protein-2 (or MIP-2) family of cytokines. These include MIP-2, gro (or melanoma growth-stimulatory activity), and NAP-2. See S. D. Wolpe and A. Cerami, FASEB J. 3, 2565 (1989); B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggiolini, J. Exp. Med. 171, 1797 (1990); and A. Walz, B. Dewald, V. von Tscharner, M. Baggiolini, ibid. 170, 1745 (1989). High affinity binding sites for IL-8 have been found on transformed myeloid precursor cells such as HL60 and THP-1 as well as on neutrophils. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991); J. Besemer, A. Hujber, B. Kuhn, J. Biol. Chem. 264, 17409 (1989); P. M. Grob et al., ibid. 265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K. Matsushima, J. Exp. Med. 169, 1185 (1989); E. J. Leonard et al., J. Immunol. 144, 1323 (1989). NAP-2 and gro compete with IL-8 for binding to human

neutrophils suggesting that they interact with the same receptors. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991).

5 Functional expression in the Xenopus oocyte has established the identity of cDNA clones encoding rabbit and human forms of another peptide chemoattractant receptor on neutrophils, the N-formyl peptide receptor. See K. M. Thomas, H. Y. Pyun, J. Navarro, J. Biol. Chem. 265, 20061 (1990);
10 and F. Boulay, M. Tardif, L. Bouchon, P. Vignais, Biochem. Biophys. Res. Commun. 168, 1103 (1990). Yet the amino acid sequence of the rabbit form of the receptor (originally designated F3R) is only 28%
15 identical with that of the human form (designated in this paper as FPR); this is far greater than the differences between species reported for all other G protein-coupled receptors. See T. I. Bonner, A. C. Young, M. R. Brann, N. J. Buckley, Neuron 1, 403
20 (1988); and S. Yokoyama, K. E. Eisenberg, A. F. Wright, Mol. Biol. Evol. 6, 342 (1989).

 By cloning the complementary DNA sequence encoding the human interleukin 8 receptor (IL8R), the primary structure of this receptor can be
25 established and its role in the inflammatory response can be further investigated. Such studies could potentially lead to the design of new anti-inflammatory agents.

SUMMARY OF THE INVENTION

It is an object of the present invention to isolate and characterize human interleukin-8 receptors.

5 The present invention relates to a cDNA clone from HL60 neutrophils, designated p2, which encodes an IL-8 receptor having an amino acid sequence as shown in Figure 3.

10 The present invention further relates to the IL-8 receptor itself which has intracellular calcium store mobilizing properties and ligand binding properties.

15 Furthermore, the invention relates to an oocyte expressing IL-8 receptors produced by injecting a cRNA molecule transcribed from the cDNA clone, p2. Additionally, the present invention relates to a method of producing the IL-8 receptor in an oocyte.

20 Furthermore, the present invention relates to a host cell stably transfected with the cDNA clone, p2. In addition, the present invention relates to a method of producing the IL-8 receptor in a host cell.

25 In addition, the present invention relates to a method of detecting the presence or absence of a DNA segment encoding the IL-8 receptor in a sample by contacting the sample with a DNA probe having at least a portion of the sequence of the cDNA clone, p2.

Various other objects and advantages of the present invention will become apparent from the following figures and description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Fig. 1 shows the results of in vitro translation of p2 and distribution of p2 mRNA in various cell types.

 Fig. 1 (A) shows translation of p2 cRNA by rabbit reticulocyte lysate (lane 2) compared with control lysate to which no RNA was added (lane 1). Red blood cell membrane proteins were used as molecular mass standards indicated in kilodaltons (kD) at the left. The gel was exposed to XAR-2 film for 12 hours.

15 Fig. 1 (B) shows p2 mRNA distribution. The blot of RNA from neutrophils was prepared separately from 10 μ g of total cellular RNA. The other lanes derive from a single blot containing total cellular RNA from peripheral blood T lymphocytes activated with phytohemagglutinin (PHA-T, 5 μ g), THP-1 cells (5 μ g) and Jurkat cells (3 μ g). The lane marked HL60 contains 10 μ g of polyadenylated [poly (A)] RNA from undifferentiated HL60 cells. The arrow indicates the location of a faint band of RNA from THP-1 cells. Both blots were hybridized under identical conditions with the same p2 probe and were

20

25

washed at 68°C in 0.1 X SSPE for 1 hour. Blots were exposed to XAR-2 film in a Quanta III cassette at -80°C for 5 days. Results with 3 independent HL60 cell preps and 2 separate THP-1 and neutrophil blots were identical.

Fig. 2 shows the expression of a human IL-8 receptor in Xenopus oocytes.

Fig. 2 (A) shows signal transduction by the IL-8 receptor. Four days after injection with 5 ng of p2 cRNA, oocytes were stimulated with the indicated concentration of IL-8 and calcium efflux was measured. Data derive from five replicate determinations per point and are representative of three separate experiments.

Fig. 2 (B) shows binding of [¹²⁵I]-IL-8 to oocytes expressing a functional IL-8 receptor. Total (○) and non-specific binding (○) was determined by incubating oocytes injected with p2 cRNA with the indicated concentration of radioligand in the absence or presence of unlabeled IL-8 (1 μM), respectively. The data shown are the mean ± SEM of triplicate determinations per point and are representative of two separate experiments. Non-specific binding was subtracted from total binding to determine specific binding (□). C5a (1 μM) did not displace [¹²⁵I]-IL-8 from oocytes injected with p2 cRNA. Specific binding of [¹²⁵I]-IL-8 by oocytes injected with water was undetectable.

Fig. 2 (C) shows ligand selectivity of the IL-8 receptor. Three days after injection with 5 ng of p2 cRNA, oocytes were stimulated with the indicated concentration of IL-8 (O), gro (\square), NAP-2 (O), FMLP (\blacklozenge) or C5a (Δ), and calcium efflux activity was measured. Data derive from eight replicate determinations per point. The response of oocytes injected with 50 ng of HL60 neutrophil RNA to FMLP ($1\ \mu\text{M}$) or C5a ($500\ \text{nM}$) was 51 ± 3 and $16 \pm 5\%$, respectively. The response of oocytes injected with 5 ng of an irrelevant cRNA encoding the rat serotonin $1c$ receptor was negligible for each of the five ligands; the response to the relevant ligand, serotonin ($1\ \mu\text{M}$), was $34 \pm 3\%$ ($n = 6$). See D. Julius, A. B. McDermott, R. Axel, T. M. Jessell, Science 241, 558 (1988). In (A) and (C) basal amounts of calcium efflux and calcium uptake were similar among all experimental conditions.

Fig. 3 shows the primary structure of a human IL-8 receptor (IL-8R) and its alignment with that of the reported rabbit (F3R) and human (FPR) N-formyl peptide receptors. Vertical bars indicate identical residues for each adjacent sequence position. Shaded boxes indicate the location of predicted membrane spanning segments I through VII as determined by the Kyte-Doolittle algorithm. See J. Kyte and R. F. Doolittle, J. Molec. Biol. 157, 105 (1982). Open boxes designate predicted sites for N-

linked glycosylation. Arabic numbers above the sequence blocks enumerate the IL-8 receptor sequence and are left justified. Dashes indicate gaps that were inserted to optimize the alignment.

5 Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gly; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

10 Fig. 4 relates to a genomic analysis of a human IL-8 receptor. A Nytran blot of human genomic DNA digested with the indicated restriction endonucleases was hybridized with full-length cDNA of the IL-8 receptor at high stringency (final wash
15 at 68°C in 0.1 X SSPE for 1 hour). The blot was exposed to Kodak XAR-2 film in a Quanta III cassette at -80°C for 5 days. The position of chain length standards is indicated in kilobases at the left. The autoradiogram shown is representative of two
20 independent experiments.

Fig. 5 shows the nucleotide sequence of p2, having a length of 1510 nucleotides.

Fig. 6 shows the oligonucleotide probe corresponding to nucleotides 238 to 276 of the cDNA
25 sequence of F3R.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a human IL-8 receptor and its encoding cDNA clone, designated p2. This receptor is a newly identified human homologue of F3R.

5 The present invention further relates to a cRNA molecule transcribed from the cDNA clone, p2. The present invention also relates to an interleukin-8 receptor which is also a gro receptor and has greater than 77% homology with the amino acid sequence shown in Figure 3.

10 Furthermore, the present invention relates to an oocyte, such as a Xenopus laevis oocyte, which expresses a functionally active form of the IL-8 receptor, when it is injected with a cRNA molecule transcribed from the cDNA clone, p2. The invention also relates to the method of injecting p2 into individual oocytes using standard techniques and experimental conditions which would be understood by one skilled in the art.

20 Additionally, the present invention relates to a recombinant DNA molecule and to a host cell transfected therewith which expresses a functionally active form of the IL-8 receptor. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and a cDNA segment, p2, can be constructed using methods known in the art without undue experimentation. The transfected host

25

cell can be cultured, and the expressed protein can be isolated and obtained in a substantially pure form using methods known in the art. COS cells (monkey kidney cells) or NIH 3T3 or other eukaryotic host cells conventionally used in the art to express inserted cDNA may also be used. As a vector, pCDNAI or pCLNXneo can be used as well as other vectors conventionally used in the art.

The invention further relates to the IL-8 receptor's intracellular calcium store mobilizing properties and ligand binding properties. Specifically, the IL-8 receptor encoded by p2 has been shown to bind both IL-8 and gro and to exhibit calcium flux as a result of such binding.

The invention also relates to a method of detecting the presence or absence of a DNA segment encoding the IL-8 receptor or a related receptor from the MIP-2 family in a sample by contacting the sample with a p2 probe having at least a portion of the cDNA clone. The method is performed under conditions such that hybridization between the probe and the DNA segment from the sample occurs. This hybridization can be detected by assaying for the presence or absence of a complex formed between the probe and the DNA segment. The techniques and experimental conditions used would be understood by one skilled in the art.

The invention also relates to a method of screening ligands of the IL-8 receptor by measuring binding affinity and calcium flux resulting from the binding of the ligand to the receptor expressed in the oocyte or the host cell. The techniques and experimental conditions used would be understood by one skilled in the art.

The invention also relates to a gene therapy treatment by which an individual with a condition relating to a deficiency of IL-8 receptor might be treated by administering to the individual DNA encoding the IL-8 receptor in a form such that the DNA would alleviate the deficiency of IL-8 receptor. Conventional gene therapy techniques understood by one skilled in the art could be used. However, there is so far no evidence which points to the existence of such a condition of IL-8 receptor deficiency.

The following non-limiting examples are provided to further describe the present invention.

EXAMPLES

Example 1

The DNA segment which encodes the IL-8 receptor was obtained in the form of the cDNA clone, p2, using the following techniques and conditions.

cdNA libraries were constructed in the vector
UniZAP (Stratagene, La Jolla, CA) from 2-kb and 3.5-
kb fractions of poly(A)+ RNA from HL60 neutrophils
that had been separated on a sucrose gradient as
described in P. M. Murphy, E. K. Gallin, H. L.
Tiffany, J. Immunol. 145, 2227 (1990).

Approximately 3×10^5 plaque-forming units (pfu)
from the 2-kb library were screened with the ^{32}P -
labeled F3R oligonucleotide probe.

Both the 2-kb (3×10^5 pfu) and the 3.5-kb (10^6
pfu) libraries were rescreened under conditions of
low stringency with a ^{32}P -labeled probe of p2 cdNA
synthesized from random primers. The final wash was
for one hour at 55°C in 5 X SSPE (1 X SSPE contains
150 mM NaCl, 10 mM NaH_2PO_4 , and 1 mM Na_2EDTA , pH 7.4).

The DNA sequence was determined with sequence-
based oligonucleotides (17 bases) by the
dideoxynucleotide chain termination method. See F.
Sanger, S. Nicklen, A. R. Coulson, Proc. Natl. Acad.
Sci. U.S.A. 74, 5463 (1977).

DNA sequences were analyzed using software from
the University of Wisconsin Genetics Computer Group
on a Cray supercomputer maintained by the National
Cancer Institute Advanced Scientific Computing
Laboratory, Frederick Cancer Research Facility,
Frederick, MD. See J. Devereux, P. Haeberli, O.
Smithies, Nucleic Acids Res. 12, 389 (1984).

An oligonucleotide probe corresponding to nucleotides 238 to 276 of the cDNA sequence of F3R (Fig. 6) was hybridized to cDNA libraries made from RNA from the promyelocytic leukemia cell line HL60 grown for two days in the presence of dibutyryl cyclic adenosine monophosphate (750 μ M), a treatment that induces a neutrophil-like phenotype. See K. M. Thomas, H. Y. Pyun, J. Navarro, J. Biol. Chem. 265, 20061 (1990). Seven clones that encoded an identical gene product were isolated. The longest of these, designated p2, was sequenced on both strands (Fig. 5). Confirmatory sequences were obtained from the other clones. A 1065-bp (base pair) open reading frame begins with the sequence AACATGG which conforms to the Kozak consensus criteria for translation initiation sites. See M. Kozak, Nucleic Acids Res. 15, 8125 (1987). A 24 bp poly(A) tail is found at the end of a 405 bp 3'-untranslated region.

The cDNA clone, p2, which was obtained was characterized and found to encode the IL-8 receptor.

Example 2

p2 cRNA was synthesized by in vitro transcription with T3 RNA polymerase of a pBluescript construct that had been cleaved with Xho I. p2 cRNA (500 ng) was incubated for 30 min at 30°C with rabbit reticulocyte lysate and [³⁵S]-methionine

in a 25 μ l reaction volume (Promega, Madison, WI).
Labeled proteins (40% of the yield) were then
separated by SDS-polyacrylamide gel electrophoresis
[10% gels (Novex, Encinitas, CA)]. The gel was
5 stained with Coomassie blue, fixed, impregnated with
Fluoro-Hance (Research Products International, Mount
Prospect, ILL), and dried before autoradiography.

It has been found that RNA synthesized in vitro
from p2 cDNA (p2 cRNA) directed the synthesis of a
10 polypeptide of 32 kilodaltons in vitro (Fig. 1A).
This is the size of the deglycosylated native N-
formyl peptide receptor as well as the size found
for FPR protein synthesized in vitro. See H. L.
Malech, J. P. Gardner, D. F. Heiman, S. A.
15 Rosenzweig, J. Biol. Chem. 260, 2509 (1985). It is
known that binding sites for N-formyl peptides are
expressed in mature but not in immature myeloid
cells. See R. Sullivan, J. D. Griffin, H. L.
Malech, Blood 70, 1222 (1987). It is also known
20 that expression of RNA for FPR is restricted to
mature myeloid cells as well.

In contrast, it has been found that when p2 was
used as a probe, it hybridized with a single 3-kb
band on blots of RNA from the myeloid precursor cell
25 lines HL60 and THP-1, and from normal blood-derived
human neutrophils, but not from peripheral blood T
lymphocytes or Jurkat cells (Fig. 1B). (For a
description of RNA preparation and blot

hybridization, see P. M. Murphy and H. L. Tiffany,
J. Biol. Chem. 265, 11615 (1990).) This pattern of
expression of p2 RNA is more like the distribution
of IL-8 binding sites than N-formyl peptide binding
5 sites. See J. Besemer, A. Hujber, B. Kuhn, J. Biol.
Chem. 264, 17409 (1989); P. M. Grob et al., ibid.
265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K.
Matsushima, J. Exp. Med. 169, 1185 (1989); and E. J.
Leonard et al., J. Immunol. 144, 1323 (1989).

10. Example 3

The materials and methods used for the calcium
efflux assay were as described in P. M. Murphy, E.
K. Gallin, H. L. Tiffany, J. Immunol. 145, 2227
(1990). Oocytes were microinjected with RNA samples
15 in a total volume of 50 nl per oocyte 3 days after
harvesting and were then incubated at 20 to 23°C for
2 to 4 days. Oocytes were then incubated with "Ca"
[50 µCi/ml (ICN Biomedicals, Costa Mesa, CA)] for 3
hours. After ten washes with medium, individual
20 oocytes were stimulated with ligand in wells of a
96-well tissue culture plate containing 100 µl of
medium. Three 100 µl samples of the incubation
medium were collected and analyzed by liquid
scintillation counting: a) the final 100 µl wash (20
25 min) before application of ligand; b) fluid
containing the stimulus, removed after a 20 min
incubation with the oocyte; and c) the oocyte

solubilized in SDS (1%) in medium 20 min after stimulation. Data are presented as the mean \pm standard error of the mean (SEM) of the percent of loaded $^{45}\text{Ca}^{2+}$ that was released by individual oocytes in response to the stimulus, or $[(b-a) \div (b+c)] \times 100$. FMLP and recombinant human C5a was from Sigma, St. Louis, MO. Recombinant human IL-8 was from Genzyme, Boston, MA. Recombinant human NAP-2 was from Bachem, Philadelphia, PA.

IL-8 was iodinated to a specific activity of 260 Ci/mmol as described in H. L. Malech, J. P. Gardner, D. F. Heiman, S. A. Rosenzweig, J. Biol. Chem. 260, 2509 (1985). The radioligand was qualified by binding to human neutrophils. Single oocytes were incubated with ^{125}I -IL-8 for 30 min on ice in 10 μl binding buffer (Hanks balanced salt solution with 25 mM HEPES, 1% bovine serum albumin, pH 7.4). Unbound ligand was removed by pelleting the oocyte through 300 μl F50 silicone fluid (General Electric, Waterford, NY). The tube was quick-frozen and gamma emissions from the amputated tips were counted.

When Xenopus oocytes were injected with p2 cRNA, they mobilized intracellular calcium in response to IL-8 with an EC_{50} of 20 nM (Fig. 2A), but did not respond to N-formyl methionyl-leucyl-phenylalanine (FMLP). This value is approximately 20-fold higher than that reported for human

neutrophils with recombinant human IL-8. See B. Moser, I. Clark-Lewis, R. Zwahlen, M. Baggiolini, J. Exp. Med. 171, 1797 (1990). The receptor specifically bound IL-8 over the same concentration range as for stimulation of calcium flux (Fig. 2B). Since specific binding did not saturate at the highest concentration of radioligand that could be meaningfully tested, a dissociation constant could not be determined. Thus the receptor encoded by p2, when expressed in the oocyte, appears to bind IL-8 with a lower affinity than do neutrophil binding sites for IL-8. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991); J. Besemer, A. Hujber, B. Kuhn, J. Biol. Chem. 264, 17409 (1989); P. M. Grob et al., ibid. 265, 8311 (1990); A. K. Samanta, J. J. Oppenheim, K. Matsushima, J. Exp. Med. 169, 1185 (1989); and E. J. Leonard et al., J. Immunol. 144, 1323 (1989).

The IL-8 receptor expressed by Xenopus oocytes also activated a calcium flux in response to structurally related ligands with a rank order of potency of IL-8 > gro > NAP-2. This is identical to the rank order of competition with [¹²⁵I]-IL-8 for binding to neutrophils. See B. Moser, C. Schumacher, V. von Tscharner, I. Clark-Lewis, M. Baggiolini, J. Biol. Chem. 266, 10666 (1991). C5a, a structurally unrelated chemoattractant that is

similar in size (74 amino acids) and charge (pI 8.6) to IL-8, did not activate the IL-8 receptor (Fig. 2C).

To further support the data obtained from the oocyte studies, the p2 cDNA was also cloned into the vector pCDNAI. When COS cells are transiently transfected with the pCDNAI-p2 construct, specific binding sites were detected with ¹²⁵I-labeled IL-8 and gro protein. In contrast to the ligand binding affinity in the oocyte environment, the affinity for the ligand when the receptor encoded by p2 was expressed in the COS cell was higher. Specifically, the K_d for IL-8 was 2 nM and the K_d for gro was 1.3 nM. These results corroborate the data obtained from the oocyte studies, indicating that p2 encodes a receptor for both IL-8 and gro.

Example 4

A p2 probe was hybridized under conditions of high stringency to blots of human genomic DNA. Specifically, human genomic DNA (3 μ g per lane) was digested with 6 units of Eco RI, Eco RV, Hind III, Pst I, or Xba I restriction endonucleases (Boehringer-Mannheim, Indianapolis, IN) and was then fractionated by electrophoresis on an agarose gel (1%).

After denaturation in alkaline solution the DNA was transferred to a Nytran filter by capillary

action. The banding pattern obtained was most consistent with one copy per haploid genome of a small gene encoding the IL-8 receptor (Fig. 4). Detection of faint bands, however, in DNA digested with Eco RV, Hind III, and Xba I after long exposure of the blot suggested that another human homologue of F3R could be found with the p2 probe. HL60 neutrophil cDNA libraries were therefore rescreened with a p2 probe. Thirteen hybridizing plaques were sequenced; all were identical to p2. Therefore, a gene encoding a receptor more closely related to F3R is expressed either at very low levels, or not at all, in HL60 neutrophils.

Characteristics of various portions of the IL-8 receptor have been established. It has been found that the receptor contains seven hydrophobic segments predicted to span the cell membrane, a characteristic of the superfamily of G protein-coupled receptors (Fig. 3). The COOH-terminal segment contains 11 serine or threonine residues that may be phosphorylation sites for cellular kinases. The 20 amino acid third cytoplasmic loop, which may interact with G proteins, is similar in size to that of other peptide receptors. The IL-8 receptor has a single predicted site for N-linked glycosylation in the NH₂-terminal segment and two sites in the second extracellular loop. As with the C5a receptor, the NH₂-terminal segment is rich in

acidic residues and may form the binding site for IL-8, which is basic (pI-9.5). See N. P. Gerard and C. Gerard, Nature 349, 614 (1991).

5 The IL-8 receptor has been compared with other related receptors. The IL-8 receptor possesses 69% amino acid identity to F3R after the imposition of 10 gaps. If only the predicted transmembrane domains (TMD) are compared, 84% identity is found with F3R. Alignment with ten other G protein-coupled receptor sequences and examination of 10 corresponding DNA sequences indicates that the apparent divergence of the IL-8 receptor from F3R between residues 92 and 105 is due to a frame shift in F3R. See T. I. Bonner, A. C. Young, M. R. Brann, 15 N. J. Buckley, Neuron 1, 403 (1988); and S. Yokoyama, K. E. Eisenberg, A. F. Wright, Mol. Biol. Evol. 6, 342 (1989). Moderately conserved domains include the NH₂-terminal segment (38% identity, 4 gaps), the first extracellular loop (33%, 1 gap) and 20 the COOH-terminal 23 residues (22%, no gaps). The third cytoplasmic loops are 95% identical. The IL-8 receptor possesses less than 30% amino acid identity with all other reported G protein-coupled receptor sequences including that of FPR (Fig. 3).

25 In a related development, a cDNA from human neutrophils has been found that encodes a distinct IL-8 receptor. This receptor has 77% amino acid identity with the IL-8 receptor encoded by p2, and

is more closely related to F3R (79% versus 69% amino acid identity). Neither human IL-8 receptor interacts with N-formyl peptides. The receptor encoded by p2 diverges most extensively from the other two sequences in the NH₂-terminal segment, although the acidic character of this region is conserved. Thus, the human neutrophil expresses at least two distinct calcium mobilizing IL-8 receptors. One of these, that encoded by p2, also is a gro receptor. The ability to bind gro of the other IL-8 receptor, the one more closely related to F3R, is not known. Structural comparison of the human IL-8 receptors with F3R predicts that F3R encodes a high affinity rabbit IL-8 receptor. In fact, to corroborate this prediction, Thomas et al. recently reported that F3R does encode a high affinity rabbit IL-8 receptor (See K.M. Thomas, L. Taylor, J. Navarro, J. Biol. Chem., 266, 14839-14841 (1991)).

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications cited in this application are specifically incorporated by

reference herein.

WHAT IS CLAIMED IS:

1. An interleukin-8 receptor wherein said receptor has an amino acid sequence as shown in Figure 3.
2. A DNA segment encoding the receptor of claim 1 wherein said receptor has a nucleotide sequence as shown in Figure 5.
3. A substantially pure form of the interleukin-8 receptor according to claim 1.
4. An interleukin-8 receptor wherein said receptor has greater than 77% homology with an amino acid sequence shown in Figure 3 and is a gro receptor.
5. A recombinant DNA molecule comprising:
 - a) said DNA segment according to claim 2; and
 - b) a vector for introducing said DNA into host cells.
6. The recombinant DNA molecule according to claim 5 wherein said vector is pCDNAI.

7. The receptor of claim 1 wherein said receptor has intracellular calcium mobilizing properties and ligand binding properties.
8. A host cell stably transfected with the recombinant DNA molecule of claim 1 in a manner allowing expression of a functionally active form of said protein encoded by said DNA segment.
9. The host cell of claim 8 wherein said host cell is a COS cell.
10. A method of producing an Interleukin-8 protein, said protein having an amino acid sequence as shown in Figure 3 comprising the steps of
 - culturing host cells according to claim 8 in a manner allowing expression of said protein and
 - isolating said protein from said host cells.
11. An RNA molecule transcribed from the DNA segment of claim 2.
12. An oocyte containing the RNA molecule of claim 11 in a manner allowing expression of a functionally active form of said receptor encoded by said DNA segment.

13. The oocyte of claim 12 wherein said oocyte is a *Xenopus laevis* oocyte.

14. A method of producing an interleukin-8 receptor protein in an oocyte comprising the steps of
injecting an oocyte with the RNA molecule of claim 11,
expressing said DNA segment in said oocyte,
and isolating said protein from said oocyte.

16. A method of detecting the presence or absence in a sample of a DNA segment encoding interleukin-8 receptor or a related MIP-2 receptor protein comprising the steps of

contacting said sample with a DNA probe comprising at least a portion of said DNA segment of claim 2 under conditions such that hybridization between said probe and said DNA segment of said sample occurs, and

detecting the presence or absence of a complex formed between said probe and said DNA segment.

17. A method of screening a ligand of the IL-8 receptor according to claim 1 comprising the steps of

contacting said ligand to the receptor under conditions such that binding to the receptor can occur,

measuring binding affinity of the ligand to the receptor and

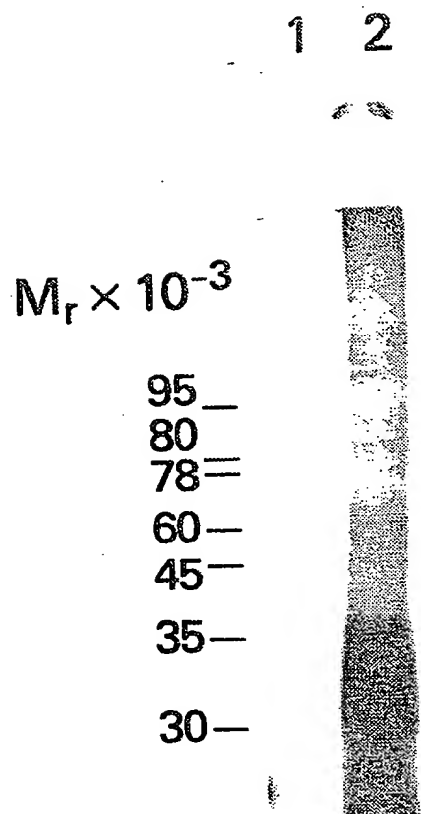
measuring calcium flux associated with the binding of the ligand to the receptor.

18. The method according to claim 17 wherein the receptor is in the oocyte according to claim 12.

19. The method according to claim 16 wherein the receptor is in the host cell according to claim 8.

1 / 11

FIG. 1A



SUBSTITUTE SHEET

2 / 11

FIG. 1B

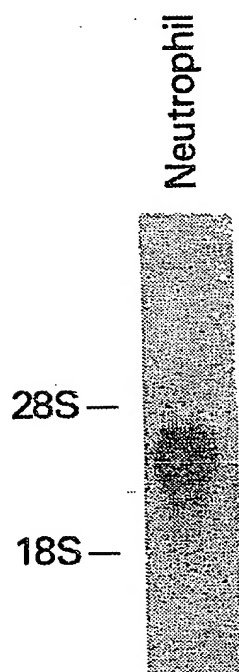
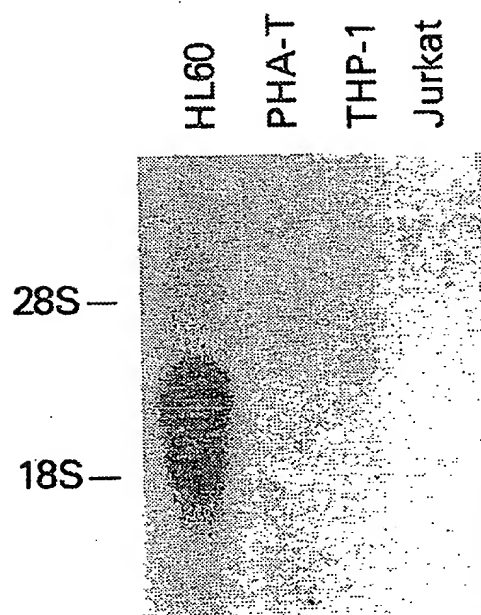


FIG. 1C



3 / 1 1

FIG. 2C

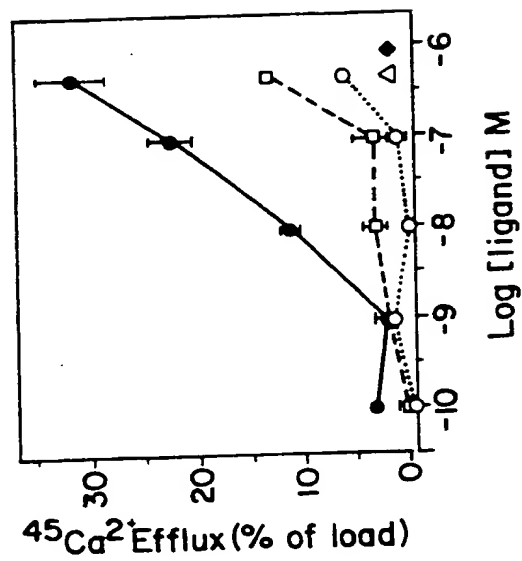


FIG. 2B

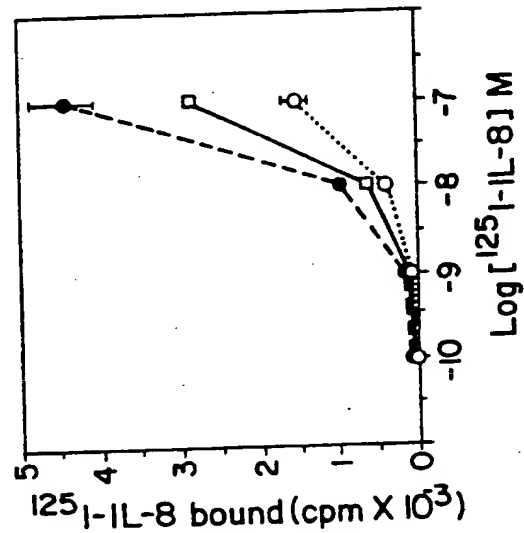
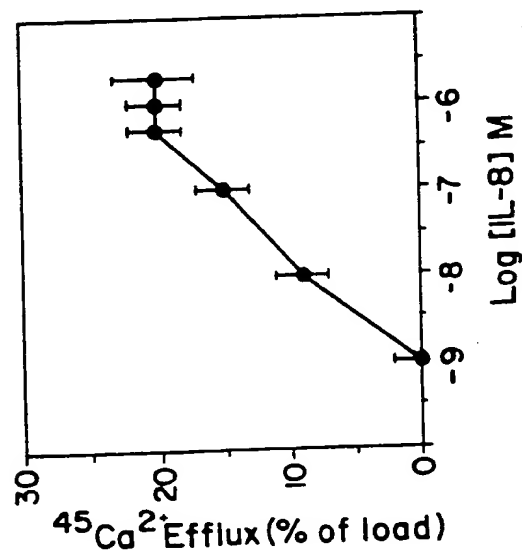


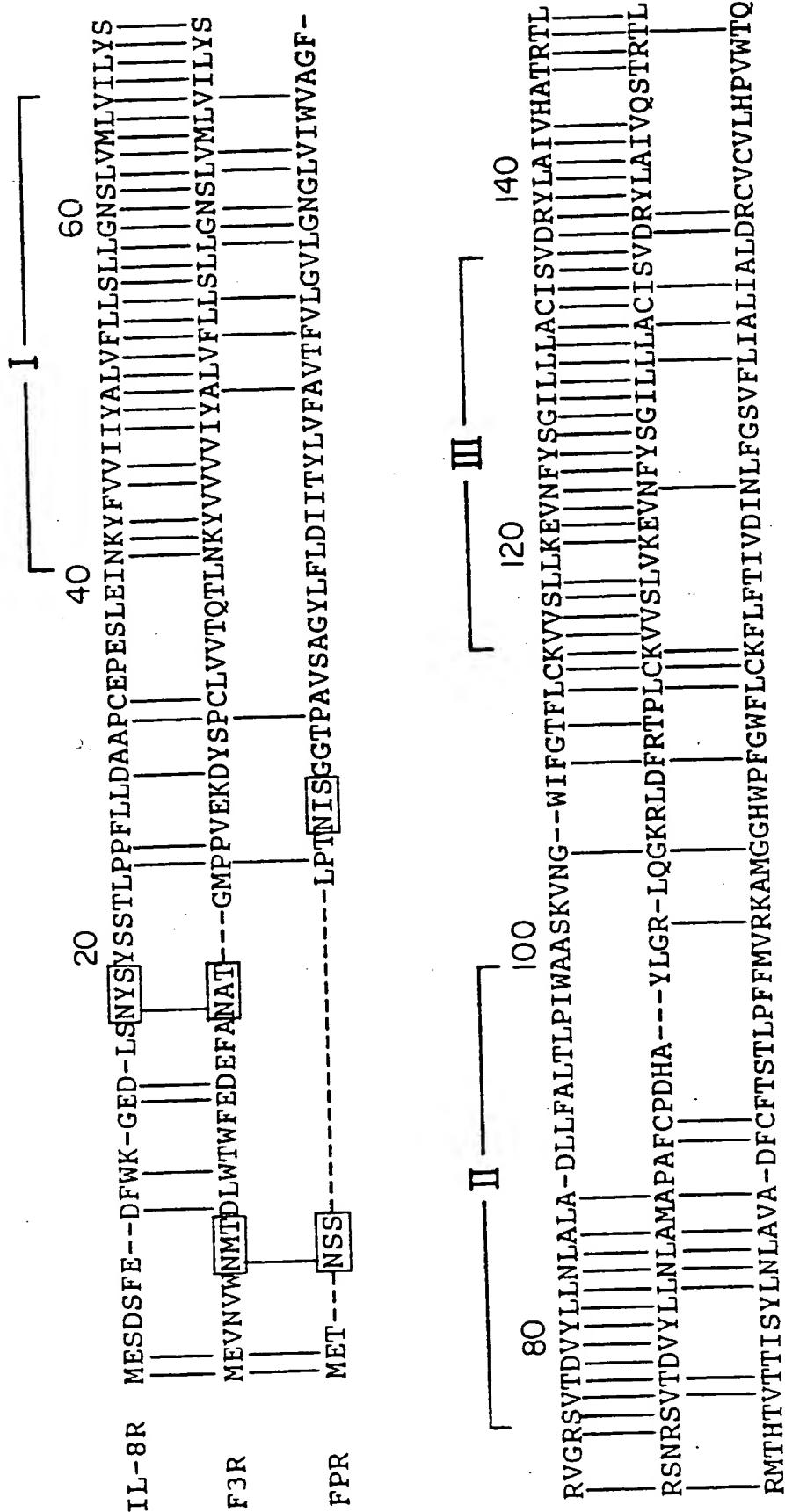
FIG. 2A



SUBSTITUTE SHEET

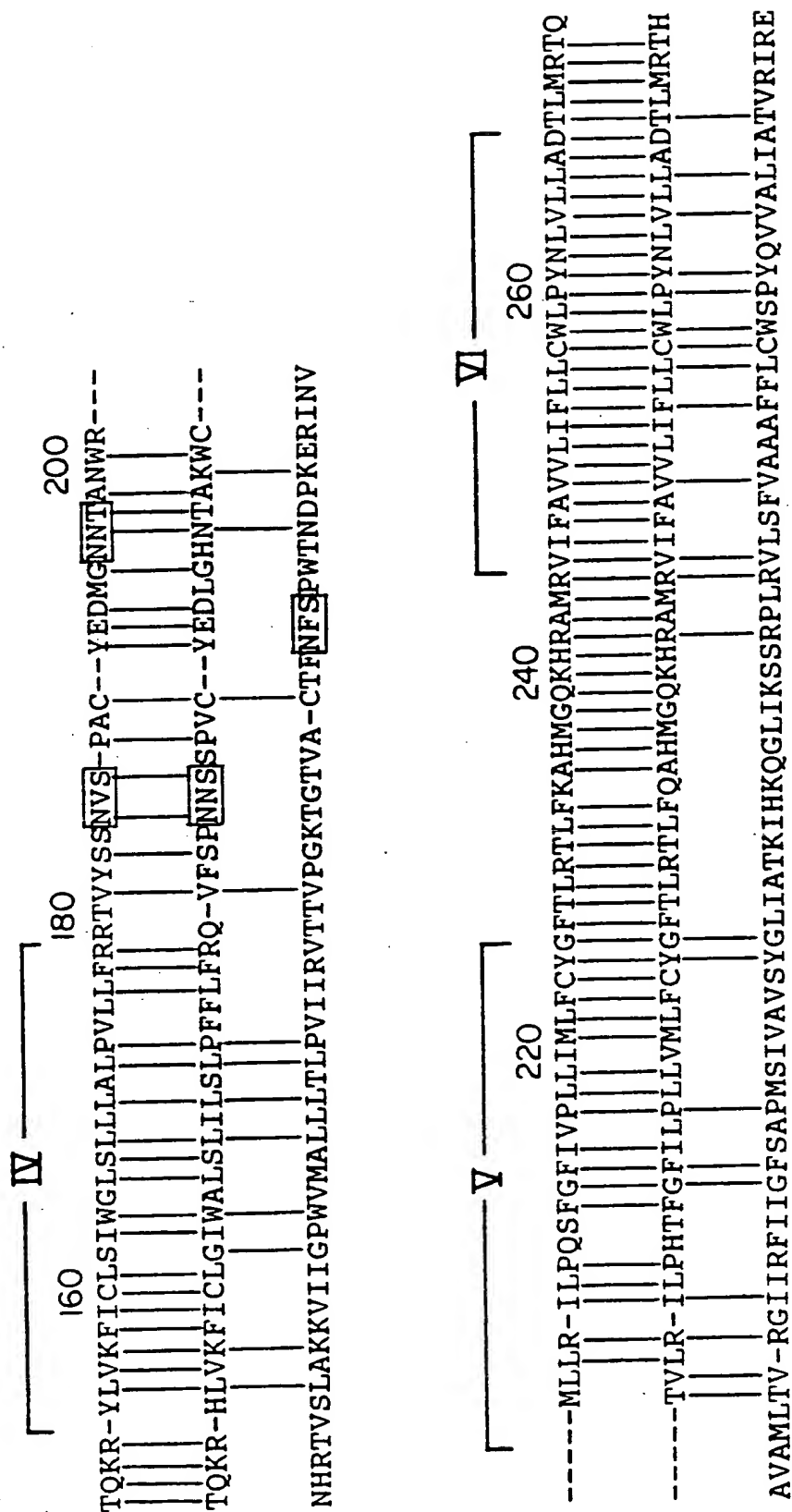
4 / 1 1

FIG. 3A



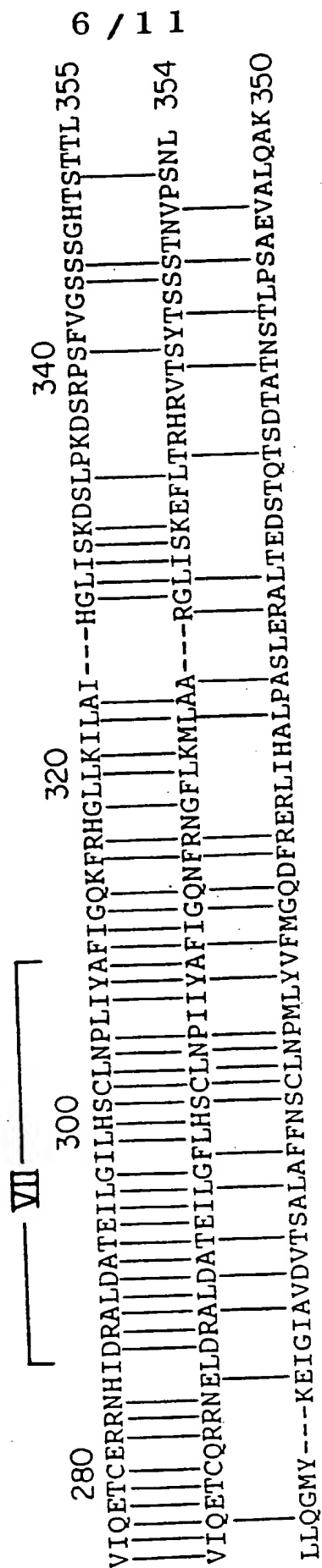
5 / 1 1

FIG. 3B



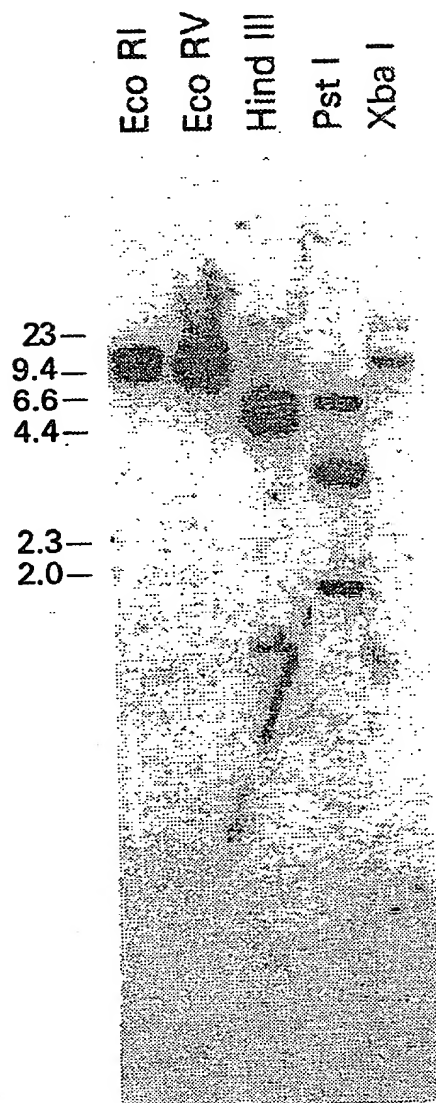
SUBSTITUTE SHEET

FIG. 3C



7 / 11

FIG. 4



SUBSTITUTE SHEET

8 / 1 1

FIG. 5A

1 GTCAGGATTT AAGTTTACCT CAAAATGGA AGATTTTAAC ATGGAGAGTG
51 ACAGCTTTGA AGATTCTGG AAAGTGAAG ATCTTAGTAA TTACAGTTAC
101 AGCTCTACCC TGCCCCCTTT TCTACTAGAT GCCGCCCCAT GTGAACCAGA
151 ATCCCTGGAA ATCAACAAGT ATTTTGTTGGT CATTATCTAT GCCCTGGTAT
201 TCCTGCTGAG CCTGCTGGA AACTCCCTCG TGATGCTGGT CATCTTATAC
251 AGCAGGGTCG GCCGCTCCGT CACTGATGTC TACCTGCTGA ACCTAGCCTT
301 GGCCGACCTA CTCTTTGCCC TGACCTTGCC CATCTGGGC GCCTCCAAGG
351 TGAATGGCTG GATTTTGGC ACATTCTGT GCAAGTGTTG CTCACTCCTG
401 AAGGAAGTCA ACTTCTATAG TGGCATCCTG CTA CTGGCCT GCATCAGTGT
451 GGACCGTTAC CTGGCCATTG TCCATGCCAC ACGCACACTG ACCCAGAAGC

9 / 1 1

FIG. 5B

501 GCTACTTGGT CAAATTGATA TGTCTCAGCA TCTGGGGTCT GTCCCTTGCTC
551 CTGGCCCTGC CTGTCTTACT TTTCCGAAGG ACCGTCTACT CATCCAATGT
601 TAGCCCAGCC TGCTATGAGG ACATGGGCAA CAATACAGCA AACTGGCGGA
651 TGCTGTTACG GATCCTGCCC CAGTCCTTTG GCTTCATCGT GCCACTGCTG
701 ATCATGCTGT TCTGCTACGG ATTACCCCTG CGTACGCTGT TTAAGGCCCA
751 CATGGGGCAG AAGCACCGGG CCATGCGGGT CATCTTTGCT GTCGTCCTCA
801 TCTTCCCTGCT TTGCTGGCTG CCTACAACC TGGTCCTGCT GGCAGACACC
851 CTCATGAGGA CCCAGGTGAT CCAGGAGACC TGTGAGCGCC GCAATCACAT
901 CGACCGGGCT CTGGATGCCA CCGAGATTCT GGCATCCTT CACAGCTGCC
951 TCAACCCCTT CATCTACGCC TTCAATTGGCC AGAAGTTTCG CCATGGACTC

10 / 11

FIG. 5C

1001 CTCAAGATTC TAGCTATACA TGGCTTGATC AGCAAGGACT CCCTGCCCAA
1051 AGACAGCAGG CCTTCCTTTG TTGGCTCTTC TTCAGGGGCAC ACTTCCACTA
1101 CTCTCTAAGA CCTCCTGCCT AAGTGCAGCC CGTGGGGTTC CTCCTTCTTC
1151 TTCACAGTCA CATTCCAAGC CTCATGTCCA CTGGTTCTTC TTGGTCTCAG
1201 TGTCAATGCA GCCCCCATTG TGGTCACAGG AAGCAGAGGA GGCCACGTTTC
1251 TTAGTAGTTT CCCTTGCGATG GTTTAGAAAG CTTGCCCCCTGG TGCCTCACCC
1301 CTTGCCATAA TTAGTATGTC ATTTGCTGGA GCTCTGCCCA TCCTGCCCTT
1351 GAGCCCATGG CACTCTATGT TCTAAGAAGT GAAAATCTAC ACTCCAGTGA
1401 GACAGCTCTG CATACTCATT AGGATGGCTA GTATCAAAAG AAAGAAAATC
1451 AGGCTGGCCA ACGGGATGAA ACCCTGTCTC TACTAAAAAT ACAAAAAAAA
1500 AAAAAAAAAA

11 / 11

FIG. 6

5'-GACGTCTACCTGCTGAACCTGGCCATGGCACCTGCTTTT-3'

INTERNATIONAL SEARCH REPORT

PCT/US92/07641

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12P 21/06; C12Q 1/68; C07K 3/00, 13/00, 15/00, 17/00; G01N 33/566
US CL : 435/6, 69.1, 320.1; 436/501; 530/351; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 320.1; 436/501; 530/351; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Intelligenetics, GenBank

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Biol. Chem., Vol. 265, No. 14, issued 15 May 1990, P.M. Grob et al., "Characterization of a receptor for human monocyte-derived neutrophil chemotactic factor/interleukin-8", pages 8311-8316, see entire document.	1-11, 16, 17
T	Science, Vol. 253, issued 13 September 1991, W.E. Holmes et al., "Structure and functional expression of a human interleukin-8 receptor", pages 1278-1280, see entire document.	1-11, 16, 17
T	Science, Vol. 253, issued 13 September 1991, P.M. Murphy et al., "Cloning of complementary DNA encoding a functional human interleukin-8 receptor", pages 1280-1283, see entire document.	1-11, 16, 17
Y	J. Biol. Chem., Vol. 265, No. 20, issued 15 July 1990, P.M. Murphy et al., "Characterization of phagocyte P2 nucleotide receptors expressed in Xenopus oocytes", pages 11615-11621, see entire document.	12-14, 18



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be part of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z

document member of the same patent family

Date of the actual completion of the international search

24 NOVEMBER 1992

Date of mailing of the international search report

02 DEC 1992

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

KAREN COCHRANE CARLSON, PH.D.

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07641

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Biol. Chem., Vol. 266, No. 19, issued 05 July 1991, P.M. Murphy et al., "Functional expression of human formyl peptide receptor in <i>Xenopus</i> oocytes requires a complementary human factor", pages 12560-12567, see entire document.	12-14, 18

This Page Blank (uspio)